

What is claimed is:

10057219, 10057219

1           1. A method for determining whether a first test  
2 protein is capable of interacting with a second test  
3 protein, said method comprising:

4           (a) providing a first population of mating competent  
5 cells, wherein a plurality of the cells of said population  
6 contain:

7                   (i) a first counterselectable reporter gene  
8 operably linked to a first DNA-binding-protein recognition  
9 site; and

10                   (ii) a first fusion gene which expresses a  
11 first hybrid protein, said first hybrid protein comprising  
12 said first test protein covalently bonded to a DNA-binding  
13 moiety which is capable of specifically binding to said DNA-  
14 binding-protein recognition site;

15           (b) providing a second population of mating  
16 competent cells, wherein a plurality of the cells of said  
17 second population contain:

18                   (i) a second counterselectable reporter gene  
19 operably linked to a second DNA-binding-protein recognition  
20 site; and

21                   (ii) a second fusion gene which expresses a  
22 second hybrid protein, said second hybrid protein comprising  
23 said second test protein covalently bonded to a gene  
24 activating moiety;

25           (c) maintaining said first and said second  
26 populations of mating competent cells, independently, under  
27 conditions such that expression of said  
28 selectable/counterselectable reporter genes inhibits the  
29 growth of said cells;

30           (d) mixing said first and said second populations of  
31 mating competent cells under conditions conducive to  
32 formation of mated cells; and

33 (e) detecting expression of a reporter gene as a  
34 measure of the ability of said first test protein to  
35 interact with said second test protein, wherein said  
36 reporter gene is said first or said second reporter gene or  
37 another reporter gene included in said first or said second  
38 mating competent cells or said mated cells, and is operably  
39 linked to either said first or second DNA-binding-protein  
40 recognition sites.

1 2. The method of claim 1, wherein said first test  
2 protein comprises a randomly generated peptide sequence.

1 3. The method of claim 1, wherein said second test  
2 protein comprises a randomly generated peptide sequence.

1 4. The method of claim 1, wherein said first test  
2 protein comprises an intentionally designed sequence.

1 5. The method of claim 1, wherein said second test  
2 protein comprises an intentionally designed sequence.

1 6. The method of claim 1, wherein said populations  
2 of cells are yeast cells.

1 7. The method of claim 6, wherein said yeast is *S.*  
2 *cerevisiae*.

1 8. The method of claim 7, wherein one said  
2 population of cells is of the MAT $\alpha$  mating type and the other  
3 said population of cells is of the MAT $\alpha$  mating type.

1 9. The method of claim 1, wherein said first and  
2 second counterselectable reporter genes are selected from  
3 the group consisting of *URA3*, *LYS2*, and *GAL1*.

1 10. The method of claim 1, wherein said DNA-binding  
2 moiety comprises the DNA-binding domain of a protein  
3 selected from the group consisting of *GAL4*, *LexA*, and *Ace1*.

1 11. The method of claim 1, wherein said gene  
2 activating moiety comprises the transcription activation  
3 domain of a protein selected from the group consisting of  
4 *GAL4*, *VP16*, and *Ace1*.

1 12. The method of claim 1, wherein said first and  
2 second DNA-binding-protein recognition sites comprise at  
3 least one binding site for a protein selected from the group  
4 consisting of *GAL4*, *LexA*, and *Ace1*.

1 13. The method of claim 1, wherein the number of  
2 each of said first and second DNA-binding-protein  
3 recognition sites is between 1 and 20.

1 14. The method of claim 1, wherein said  
2 counterselectable gene is integrated into the genome of said  
3 mating competent or mated cells.

1 15. The method of claim 1, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 promoter which carries an upstream repressing sequence.

1 16. The method of claim 15, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 *SPO13* promoter.

1 17. The method of claim 1, wherein said expression  
2 of said counterselectable reporter gene is detected as  
3 inhibition of cell growth.

1 18. A method for determining whether a test  
2 compound is capable of disrupting binding between a first  
3 test protein and a second test protein, said method  
4 comprising:

5 (a) providing a cell containing:

6 (i) a counterselectable reporter gene operably  
7 linked to a DNA-binding-protein recognition site;

8 (ii) a first fusion gene expressing a first  
9 hybrid protein comprising said first test protein covalently  
10 bonded to a DNA-binding moiety which is capable of  
11 specifically binding to said DNA-binding-protein recognition  
12 site; and

13 (iii) a second fusion gene expressing a second  
14 hybrid protein comprising said second test protein  
15 covalently bonded to a gene activating moiety, wherein said  
16 second test protein binds said first test protein in the  
17 absence of said test compound;

18 (b) contacting said cell with said test compound  
19 under conditions such that expression of said  
20 counterselectable reporter gene inhibits cell growth; and

21 (c) detecting inhibition of expression of said  
22 counterselectable reporter gene as a measure of the ability  
23 of said compound to disrupt said binding between said first  
24 and said second test proteins.

1 19. The method of claim 18, wherein expression of  
2 said reporter gene is detected by detecting growth of said  
3 cell.

1           20. The method of claim 18, wherein said test  
2 compound is a protein.

1           21. The method of claim 20, wherein said protein  
2 which is encoded by a nucleic acid contained within a  
3 nucleic acid library.

1           22. The method of claim 20, wherein said protein  
2 comprises a randomly generated peptide sequence.

1           23. The method of claim 18, wherein said first test  
2 protein is cJun and said second test protein is selected  
3 from the group consisting of cFos and cJun.

1           24. The method of claim 18, wherein said first test  
2 protein is E2F1 and said second test protein is pRB.

1           25. The method of claim 18, wherein said cell is a  
2 yeast cell.

1           26. The method of claim 25, wherein said yeast is  
2 *S. cerevisiae*.

1           27. The method of claim 18, wherein said cell is  
2 treated to increase its ability to take up a test compound.

1           28. The method of claim 18, wherein said cell has a  
2 mutation which increases its ability to take up a test  
3 compound.

1           29. The method of claim 28, wherein said cell is an  
2 *erg6* mutant of *S. cerevisiae*.

1 30. The method of claim 28, wherein said cell is an  
2 *ise1* mutant of *S. cerevisiae*.

1 31. The method of claim 28, wherein said cell is an  
2 *ISE2* mutant of *S. cerevisiae*.

1 32. The method of claim 28, wherein said cell is an  
2 *srb1* mutant of *S. cerevisiae*.

1 33. The method of claim 18, wherein said  
2 counterselectable reporter gene is selected from the group  
3 consisting of *URA3*, *LYS2*, *GAL1*, *CYH2*, and *CAN1*.

1 34. The method of claim 18, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 promoter which carries an upstream repressing sequence.

1 35. The method of claim 34, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 *SPO13* promoter.

1 36. The method of claim 18, wherein said DNA-  
2 binding-protein recognition site comprises at least one  
3 binding site for a protein selected from the group  
4 consisting of *GAL4*, *LexA*, and *Acl1*.

1 37. The method of claim 18, wherein the number of  
2 said DNA-binding-protein recognition sites is between 1 and  
3 20.

1 38. The method of claim 18, wherein said DNA-  
2 binding moiety comprises the DNA-binding domain of a protein  
3 selected from the group consisting of *GAL4*, *LexA*, and *Acl1*.

1           39. The method of claim 18, wherein said gene  
2 activating moiety comprises the transcription activation  
3 domain of a protein selected from the group consisting of  
4 GAL4, VP16, and Ace1.

1           40. A method for determining whether a first test  
2 protein is capable of interacting with a second test protein  
3 and incapable of interacting with a third test protein, said  
4 method comprising:

5           (a) providing a cell which contains:

6               (i) a first fusion gene which expresses a first  
7 hybrid protein, said first hybrid protein comprising said  
8 first test protein covalently bonded to a gene activating  
9 moiety;

10              (ii) a reporter gene operably linked to a first  
11 DNA-binding-protein recognition site;

12              (iii) a second fusion gene which expresses a  
13 second hybrid protein, said second hybrid protein comprising  
14 said second test protein covalently bonded to a first DNA-  
15 binding moiety which is capable of specifically binding to  
16 said first DNA-binding-protein recognition site and which is  
17 incapable of specifically binding to a second DNA-binding-  
18 protein recognition site;

19              (iv) a counterselectable reporter gene operably  
20 linked to said second DNA-binding-protein recognition site; and

21              (v) a third fusion gene which expresses a third  
22 hybrid protein, said third hybrid protein comprising said  
23 third test protein covalently bonded to a second DNA-  
24 binding-moiety which is capable of specifically binding to  
25 said second DNA-binding-protein recognition site and which  
26 is incapable of binding to said first DNA-binding-protein  
27 recognition site;



28 (b) maintaining said cell under conditions such that  
29 expression of said reporter gene does not inhibit growth of  
30 said cell and expression of said counterselectable reporter  
31 gene inhibits growth of said cell; and

32 (c) detecting growth of said cell and expression of  
33 said selectable reporter gene as a measure of the ability of  
34 said first test protein to interact with said second test  
35 protein and the inability of said first test protein to  
36 interact with said third test protein.

1 41. The method of claim 40, wherein the ability of  
2 said first test protein to interact with said second test  
3 protein and not with said third test protein is measured in  
4 the presence of a test compound.

1 42. The method of claim 40, wherein said first test  
2 protein comprises a randomly generated peptide sequence.

1 43. The method of claim 40, wherein said cell is a  
2 yeast cell.

1 44. The method of claim 43, wherein said yeast is  
2 *S. cerevisiae*.

1 45. The method of claim 40, wherein said  
2 counterselectable reporter gene is selected from the group  
3 consisting of *URA3*, *LYS2*, *GAL1*, *CYH2*, and *CAN1*.

1 46. The method of claim 40, wherein said reporter  
2 gene is selected from the group consisting of *LEU2*, *TRP1*,  
3 *HIS3*, and *LacZ*.

1 47. The method of claim 40, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 promoter which carries an upstream repressing sequence.

1 48. The method of claim 40, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 *SP013* promoter.

1 49. The method of claim 40, wherein said DNA-  
2 binding-protein recognition site comprises at least one  
3 binding site for a protein selected from the group  
4 consisting of GAL4, LexA, and Ace1.

1 50. The method of claim 40, wherein the number of  
2 each of said first and second DNA-binding-protein  
3 recognition sites is between 1 and 20.

1 51. The method of claim 40, wherein said DNA-  
2 binding moiety comprises the DNA-binding domain of a protein  
3 selected from the group consisting of GAL4, LexA, and Ace1.

1 52. The method of claim 40, wherein said gene  
2 activating moiety comprises the transcription activation  
3 domain of a protein selected from the group consisting of  
4 GAL4, VP16, and Ace1.

1 53. A method for determining whether a first test  
2 RNA molecule is capable of interacting with a test protein,  
3 said method comprising:

4 (a) providing a first population of mating competent  
5 cells, wherein a plurality of the cells of said population  
6 contain:

7 (i) a first selectable/counters selectable  
8 reporter gene operably linked to a first DNA-binding-protein  
9 recognition site;

10 (ii) a first fusion gene which expresses a  
11 first hybrid RNA molecule, said RNA molecule comprising said  
12 test RNA molecule covalently bonded to a first non-random  
13 RNA molecule; and

14 (iii) a second fusion gene which expresses a  
15 first hybrid protein, said first hybrid protein comprising a  
16 DNA-binding moiety which is capable of specifically binding  
17 to said DNA-binding-protein recognition site, said DNA-  
18 binding moiety being covalently bonded to an RNA-binding  
19 moiety, wherein said RNA-binding moiety is capable of  
20 specifically binding to said non-random RNA molecule;

21 (b) providing a second population of mating  
22 competent cells, wherein a plurality of the cells of said  
23 population contain:

24 (i) a second selectable/counters selectable  
25 reporter gene operably linked to a second DNA-binding-  
26 protein recognition site; and

27 (ii) a third fusion gene which expresses said  
28 test protein covalently bonded to a gene activating moiety;  
29 and

30 (c) maintaining said first and said second  
31 populations of mating competent cells, independently, under  
32 conditions such that expression of said  
33 selectable/counters selectable reporter genes inhibits growth  
34 of the cells of said populations;

35 (d) mixing said first and said second populations of  
36 mating competent cells under conditions conducive to  
37 formation of mated cells; and

38 (e) detecting expression of said  
39 selectable/counters selectable reporter genes as a measure of

40 the ability of said test RNA molecule to interact with said  
41 test protein.

1 54. The method of claim 53, wherein said test RNA  
2 molecule comprises a randomly generated RNA sequence.

1 55. The method of claim 53, wherein said test  
2 protein comprises a randomly generated peptide sequence.

1 56. The method of claim 53, wherein said ability is  
2 measured in the presence of a test compound.

1 57. The method of claim 53, wherein the cells of  
2 said populations of cells are yeast cells.

1 58. The method of claim 57, wherein said yeast is  
2 *S. cerevisiae*.

1 59. The method of claim 58, wherein one population  
2 of cells is of the MAT $\alpha$  mating type and the other population  
3 of cells is of the MAT $\alpha$  mating type.

1 60. The method of claim 53, wherein said first and  
2 second counterselectable reporter genes are selected from  
3 the group consisting of *URA3*, *LYS2*, and *GAL1*.

1 61. The method of claim 53, wherein said DNA-  
2 binding moiety comprises the DNA-binding domain of a protein  
3 selected from the group consisting of GAL4, LexA, and Acl.

1 62. The method of claim 53, wherein said gene  
2 activating moiety comprises the transcription activation

3 domain of a protein selected from the group consisting of  
4 GAL4 and Ace1.

1 63. The method of claim 53, wherein said first and  
2 second DNA-binding-protein recognition sites comprise at  
3 least one binding site for a protein selected from the group  
4 consisting of GAL4, LexA, and Ace1.

1 64. The method of claim 53, wherein the number of  
2 each of said DNA-binding protein recognition sites is  
3 between 1 and 20.

1 65. The method of claim 53, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 promoter which carries an upstream repressing sequence.

1 66. The method of claim 65, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 SPO13 promoter.

1 67. The method of claim 53, wherein said expression  
2 of said counterselectable reporter gene is detected as  
3 inhibition of cell growth.

1 68. A method for determining whether a first test  
2 RNA molecule is capable of interacting with a second test  
3 RNA molecule, said method comprising:

4 (a) providing a first population of mating competent  
5 cells, wherein a plurality of the cells of said population  
6 contain:

7 (i) a first selectable/counterselectable  
8 reporter gene operably linked to a first DNA-binding-protein  
9 recognition site;

10 (ii) a first fusion gene which expresses a  
11 first hybrid RNA molecule, wherein said first hybrid RNA  
12 molecule comprises said first test RNA molecule covalently  
13 bonded to a first non-random RNA molecule; and

14 (iii) a second fusion gene which expresses a  
15 first hybrid protein, said first hybrid protein comprising a  
16 DNA-binding moiety which is capable of specifically binding  
17 to said DNA-binding-protein recognition site, said DNA-  
18 binding moiety being covalently bonded to a first RNA-  
19 binding moiety which is capable of specifically binding to  
20 said first non-random RNA molecule;

21 (b) providing a second population of mating  
22 competent cells, wherein a plurality of the cells of said  
23 population contain:

24 (i) a second selectable/counters selectable  
25 reporter gene operably linked to a second DNA-binding-  
26 protein recognition site;

27 (ii) a third fusion gene which expresses a  
28 second hybrid RNA molecule wherein said second hybrid RNA  
29 molecule comprises said second test RNA molecule covalently  
30 bonded to a second non-random RNA molecule; and

31 (iii) a fourth fusion gene which expresses a  
32 gene activating moiety covalently bonded to a second RNA-  
33 binding moiety which is capable of specifically binding to  
34 said second non-random RNA molecule; and

35 (c) maintaining said first and said second  
36 populations of mating competent cells, independently, under  
37 conditions such that expression of said counters selectable  
38 reporter genes inhibits growth of said cells;

39 (d) mixing said first and said second populations of  
40 mating competent cells under conditions conducive to  
41 formation of mated cells; and

42 (e) detecting expression of said counterselectable  
43 reporter genes as a measure of the ability of said first  
44 test RNA molecule to interact with said second test RNA  
45 molecule.

1 69. The method of claim 68, wherein said first test  
2 RNA molecule comprises a randomly generated RNA sequence.

1 70. The method of claim 68, wherein said second  
2 test RNA molecule comprises a randomly generated RNA  
3 sequence.

1 71. The method of claim 68, wherein said ability of  
2 said first and said second RNA molecules to interact is  
3 measured in the presence of a test compound.

1 72. The method of claim 68, wherein the cells of  
2 said populations of cells are yeast cells.

1 73. The method of claim 72, wherein said yeast is  
2 *S. cerevisiae*.

1 74. The method of claim 73, wherein one said  
2 population of cells is of the MAT $\alpha$  mating type and the other  
3 said population of cells is of the MAT $\alpha$  mating type.

1 75. The method of claim 68, wherein said first and  
2 second counterselectable reporter genes are selected from  
3 the group consisting of *URA3*, *LYS2*, and *GAL1*.

1 76. The method of claim 68, wherein said DNA-  
2 binding moiety comprises the DNA-binding domain of a protein  
3 selected from the group consisting of *GAL4*, *LexA*, and *Acl*.

1 77. The method of claim 68, wherein said gene  
2 activating moiety comprises the transcription activation  
3 domain of a protein selected from the group consisting of  
4 GAL4, VP16, and Acl1.

1 78. The method of claim 68, wherein said first and  
2 second DNA-binding-protein recognition sites comprise at  
3 least one binding site for a protein selected from the group  
4 consisting of GAL4, LexA, and Acl1.

1 79. The method of claim 68, wherein the number of  
2 said DNA-binding-protein recognition sites is between 1 and  
3 20.

1 80. The method of claim 68, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 promoter which carries an upstream repressing sequence.

1 81. The method of claim 80, wherein said  
2 counterselectable reporter gene is operably linked to a  
3 SPO13 promoter.

1 82. The method of claim 68, wherein said expression  
2 of said counterselectable reporter gene is detected as  
3 inhibition of cell growth.

1 83. A method for determining whether a test DNA  
2 molecule is capable of interacting with a test protein, said  
3 method comprising:

4 (a) providing a cell containing:

5 (i) a counterselectable reporter gene operably  
6 linked to said test DNA molecule;



7 (ii) a fusion gene which expresses said test  
8 protein covalently bonded to a gene activating moiety; and  
9 (b) detecting expression of said counterselectable  
10 reporter gene as a measure of the ability of said test DNA  
11 molecule to interact with said test protein.

1 84. The method of claim 83, wherein (i) the  
2 sequence of said test DNA is randomly generated and (ii) the  
3 protein comprises a randomly generated peptide sequence.

1 85. A method for identifying a mutation in a  
2 reference protein which affects the ability of the reference  
3 protein to interact with a test protein, said method  
4 comprising:

5 (a) providing a cell containing:

6 (i) a counterselectable reporter gene operably  
7 linked to a DNA-binding-protein recognition site;

8 (ii) a selectable reporter gene operably linked  
9 to a DNA-binding-protein recognition site;

10 (iii) a first fusion gene expressing a first  
11 hybrid protein, said first hybrid protein comprising said  
12 test protein; and

13 (iv) a second fusion gene expressing a second  
14 hybrid protein, said second hybrid protein comprising said  
15 candidate mutated reference protein, wherein said candidate  
16 protein is encoded within a nucleic acid library of mutant  
17 alleles of the gene encoding said reference protein, and  
18 wherein one of said first and said second  
19 hybrid proteins further comprises a DNA-binding moiety which  
20 is capable of specifically binding to said DNA-binding-  
21 protein recognition site, and the other of said first and  
22 said second hybrid proteins further comprises a gene  
23 activating moiety;

24 (b) maintaining said cell under conditions such that  
25 expression of said counterselectable reporter gene at a  
26 level equal to or greater than the level of expression  
27 obtained with said reference protein inhibits growth of said  
28 cell, and such that expression of said counterselectable  
29 reporter gene at a level less than the level of expression  
30 obtained with said reference protein does not inhibit growth  
31 of said cell; and

32 (c) in a separate step, maintaining said cell under  
33 conditions such that expression of said counterselectable  
34 reporter gene does not inhibit growth of said cell, and  
35 detecting expression of said selectable reporter gene as a  
36 measure of the ability of said test protein to interact with  
37 said candidate mutated reference protein.

1 86. The method of claim 85, further comprising  
2 comparing the sequence of said candidate mutated protein  
3 with the sequence of said reference protein as an indicator  
4 of a mutation in said reference protein which affects the  
5 ability of said reference protein to interact with said  
6 first test protein.

1 87. The method of claim 85, wherein said second  
2 fusion gene encodes a functional C-term tag, and expression  
3 of said selectable reporter gene is measured as an indicator  
4 of the presence of said functional C-term tag.

1 88. The method of claim 87, wherein said functional  
2 C-term tag comprises a binding site for pRb.

1 89. A method for identifying a conditional mutant  
2 of a reference protein with decreased ability to interact  
3 with a second protein under a first set of conditions and

4 which is capable of interacting with said second protein  
5 under a second set of conditions, said method comprising:

6 (a) providing a cell containing:

7 (i) a counterselectable reporter gene operably  
8 linked to a DNA-binding-protein recognition site;

9 (ii) a selectable reporter gene operably linked  
10 to a DNA-binding-protein recognition site;

11 (iii) a first fusion gene expressing a first  
12 hybrid protein, said first hybrid protein comprising a  
13 candidate mutated reference protein, wherein said candidate  
14 protein is encoded within a nucleic acid library of mutant  
15 alleles of the gene encoding said reference protein; and

16 (iv) a second fusion gene expressing a second  
17 hybrid protein, said second hybrid protein comprising said  
18 second protein, wherein:

19 one of said first or said second hybrid  
20 proteins comprises a DNA-binding moiety which is capable of  
21 specifically binding to said DNA-binding-protein recognition  
22 site, and

23 the other of said first or said second hybrid  
24 proteins comprises a gene activating moiety;

25 (b) maintaining said cell under conditions in which  
26 expression of said counterselectable reporter gene at a  
27 level equal to or greater than the level of expression  
28 obtained with said reference protein inhibits growth of said  
29 cell, and such that expression of said counterselectable  
30 reporter gene at a level less than the level of expression  
31 obtained with said reference protein does not inhibit growth  
32 of said cell;

33 (c) in a separate step, maintaining said cell under  
34 conditions such that expression of said counterselectable  
35 reporter gene does not inhibit growth of said cell, and  
36 detecting expression of said selectable reporter gene as a

measure of the ability of said candidate mutant protein to interact with said second protein; and

(d) in a separate step, maintaining the cells under conditions identical to those in step (c) except for one parameter, and detecting expression of said selectable reporter gene as a measure of the ability of said candidate mutant protein to interact with said second protein, said expression of said selectable reporter gene under step (c) conditions but not under step (d) conditions being indicative of said conditional mutant.

90. The method of claim 89, further comprising comparing the sequence of said candidate mutant protein with the sequence of said reference protein as a means for identifying a mutant of said reference protein which has a decreased ability to interact with said second protein under a first set of conditions and which is capable of interacting with said second protein under a second set of conditions.

91. The method of claim 89, wherein said parameter is selected from the group consisting of (i) temperature and (ii) presence of a drug.

92. A method for identifying compensatory mutations in a first and a second reference protein which allow a first and a second mutant reference protein to interact with each other but not with said second and said first reference proteins, respectively, said method comprising:

(a) providing a first population of mating competent cells, wherein a plurality of the cells of said population contain:

(i) a first counterselectable reporter gene operably linked to a DNA-binding-protein recognition site;

10 (ii) a first selectable reporter gene operably  
11 linked to a DNA-binding-protein recognition site;

12 (iii) a first fusion gene which expresses a  
13 first hybrid protein, said first hybrid protein comprising  
14 said first candidate mutant protein covalently bonded to a  
15 gene activating moiety, wherein said first candidate mutant  
16 protein is encoded within a nucleic acid library of mutant  
17 alleles of said first reference protein; and

18 (iv) a plasmid containing a first  
19 counterselectable marker, and a second fusion gene which  
20 expresses a second hybrid protein, said hybrid protein  
21 comprising said second reference protein covalently bonded  
22 to a DNA-binding moiety;

23 (b) providing a second population of mating  
24 competent cells, wherein a plurality of the cells of said  
25 population contain:

26 (i) a second counterselectable reporter gene  
27 operably linked to a DNA-binding-protein recognition site;

28 (ii) a second selectable reporter gene operably  
29 linked to a DNA-binding-protein recognition site;

30 (iii) a third fusion gene which expresses a  
31 third hybrid protein, said third hybrid protein comprising  
32 said second candidate mutant reference protein covalently  
33 bonded to a DNA-binding moiety, wherein said second test  
34 protein is encoded within a nucleic acid library of mutant  
35 alleles of said second reference protein; and

36 (iv) a plasmid containing a second  
37 counterselectable marker, and a fourth fusion gene which  
38 expresses a fourth hybrid protein, said hybrid protein  
39 comprising said first reference protein covalently bonded to  
40 a gene activating moiety;

41 (c) maintaining said first and said second  
42 populations of mating competent cells, independently, under

43 conditions such that expression of said counterselectable  
44 reporter genes at a level equal to or greater than the level  
45 of expression obtained with said first and second reference  
46 proteins inhibits growth of said cells;

47 (d) maintaining said first and said second  
48 populations of mating competent cells under conditions such  
49 that expression of said counterselectable marker inhibits  
50 growth of said cells;

51 (e) maintaining said first and said second  
52 populations of mating competent cells under conditions  
53 conducive to formation of mated cells;

54 (f) detecting expression of said selectable reporter  
55 genes as a measure of the ability of said first and said  
56 second candidate mutant proteins to interact with each other  
57 and not with said second and said first reference proteins.

1 93. The method of claim 92, further comprising  
2 comparing the sequences of said first and said second  
3 candidate mutant proteins which interact with each other  
4 with the sequences of said first and said second reference  
5 proteins as a means for identifying compensatory mutations  
6 in said first and said second reference proteins.

1 94. A yeast cell having integrated into its genome  
2 a counterselectable reporter gene which is operably linked  
3 to a promoter which comprises (i) an upstream repressing  
4 sequence and (ii) a DNA-binding-protein recognition site,  
5 wherein said yeast cell lacks

6 (i) a naturally-occurring protein which is  
7 substantially identical to the protein encoded by said  
8 counterselectable reporter gene, and

9 (ii) at least one naturally-occurring protein which,  
10 when it is expressed, confers a growth advantage on a cell  
11 containing it.

1 95. The yeast cell of claim 94, wherein said  
2 counterselectable reporter gene is selected from the group  
3 consisting of *URA3*, *LYS2*, *GAL1*, *CYH2*, and *CAN1*.

1 96. The yeast cell of claim 94, wherein said  
2 promoter is a *SPO13* promoter, and said promoter comprises at  
3 least one DNA-binding-protein-recognition site for a protein  
4 selected from the group consisting of *GAL4*, *LexA*, and *Acl*.

1 97. The yeast cell of claim 96, wherein said cell  
2 is MaV103.

1 98. The yeast cell of claim 96, wherein said cell  
2 is MaV203.

1 99. The yeast cell of claim 96, wherein said cell  
2 is MaV99.

1 100. A genetic construct comprising: (i) a yeast  
2 origin of replication; (ii) a selectable marker; (iii) a  
3 yeast promoter; (iv) a nuclear localization coding signal  
4 sequence; and (v) a bacterial origin of replication.

1 101. The genetic construct of claim 100, wherein  
2 said construct is p2.5.

1 102. A genetic construct comprising: (i) a yeast  
2 origin of replication; (ii) a selectable marker; (iii) a  
3 promoter; (iv) a bacterial origin of replication; (v) a

4 counterselectable marker; and (vi) a sequence which  
5 expresses a DNA-binding moiety.

1 103. The genetic construct of claim 102, wherein  
2 said construct is p97.CYH2.

1 104. A genetic construct comprising: (i) a yeast  
2 origin of replication; (ii) a selectable marker; (iii) a  
3 promoter; (iv) a bacterial origin of replication; (v) a  
4 counterselectable marker; and (vi) a sequence which  
5 expresses a gene activating moiety.

1 105. The genetic construct of claim 104, wherein  
2 said genetic construct is pMV257.

1 106. A genetic construct comprising a  
2 counterselectable reporter gene operably-linked to a  
3 promoter, wherein said promoter comprises (i) an upstream  
4 repressing sequence and (ii) a DNA-binding-protein  
5 recognition site.

1 107. The genetic construct of claim 106, wherein  
2 said genetic construct is SPAL:URA3.